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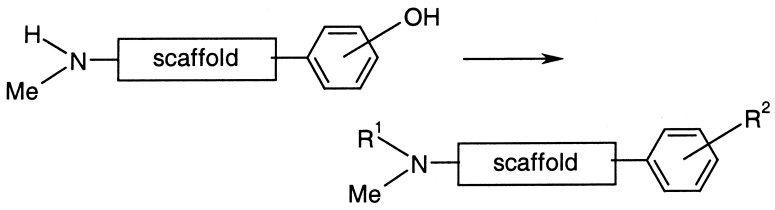

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Design and Synthesis of a Maximally Diverse and Druglike Screening Library Using REM Resin Methodology

D. Barn, W. Caulfield, P. Cowley, R. Dickins, W. Iwema Bakker, R. McGuire, J. Richard Morphy,* Z. Rankovic, and M. Thorn

Lead Discovery Chemistry, Organon Laboratories Ltd., Newhouse, ML1 5SH, Scotland, U.K.

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A 3042 compound screening library was synthesized using a combination of two solid-phase technologies: REM resin methodology and Lewis acid promoted aminolysis. The exclusivity and structural diversity of the library were enhanced by using a highly divergent synthetic strategy involving 13 different scaffolds (9 of which were custom-made), five different types of resin-bound phenol derivatization chemistry (Mitsunobu, Suzuki, acylation, sulfonylation, and carbamoylation), and three different cleavage strategies (Hofmann elimination, $AlCl_3$-promoted aminolysis, base-promoted esterolysis). This is the first example of a solid-phase Suzuki coupling involving a resin-bound aryl triflate being used for library synthesis. Computational analysis suggested that the compounds are likely to have favorable properties for CNS penetration. Analysis of the library by HPLC and MS suggested at least 90% of the sampled members were present in an average purity of ~70%. Encouragingly, hits have been identified from high-throughput screening of this library, such as compound **6**, which has an affinity of 1.02 μM for the $GlyT_2$ transporter.

Introduction

In recent years, combinatorial chemistry on solid phase has emerged as a powerful technique for the synthesis of screening libraries of small organic molecules. The aim of this project was to generate a general purpose high-quality screening library of 3000 compounds. While there is a widely held belief that screening libraries should be as chemically diverse as possible in order to increase the chances of finding activity at biologically diverse targets, we felt that incorporating functional groups that occur commonly in central nervous system (CNS)-active drugs also has merit. The frequency of structural fragments within the 373 orally bioavailable currently marketed CNS drugs is shown in Table 1.[1]

It is immediately apparent from Table 1 that aromatic rings and amines are extremely common functional groups. Since these functional groups are so common in CNS-active drugs, it was our intention that all compounds in the library should contain at least one aromatic ring and a tertiary amine group. Although we chose to bias the library toward these common functional groups, it was also our aim to increase the topological diversity of the proposed library by the use of multiple scaffolds that project these groups into different regions of space. We reasoned that the longer-term exclusivity of the library could be maximized by the use of a number of custom-made proprietary scaffolds. Synthetic feasibility was also an important consideration. Thus, we decided to use linker chemistry that our group had developed previously and that we knew to be robust: the REM resin methodology[2] and $AlCl_3$-promoted aminolysis.[3]

The library was designed around 13 scaffolds, **1** (Figure 1), each containing two sites at which diversity could be introduced, a secondary amine, and a phenol group. The phenol group is an attractive group for introducing diversity because several simple and robust transformations are possible, e.g., acylations, Mitsunobu reactions, and Suzuki couplings (via triflate). The overall strategy for the library is illustrated in Scheme 1 using one of the commercially available scaffold starting materials, *N*-methyl-(2-hydroxy)-benzylamine (**2**). Three other scaffolds, **3**−**5**, that were used were commercially available; the other nine scaffolds required a custom synthesis. Approximately 20 g of each scaffold was purchased or made. By use of this divergent

Table 1. Frequency of Functional Groups in a Group of 373 CNS Drugs

functional group	no. of drugs containing group	% occurrence (CNS drugs)	% occurrence (library)
aromatics (phenyl/benzo)	309	83	100
amines	236	63	100
amides	140	38	30
alcohols/phenols	79	21	4
esters	36	10	22
aldehydes	34	9	0
acids	16	4	0

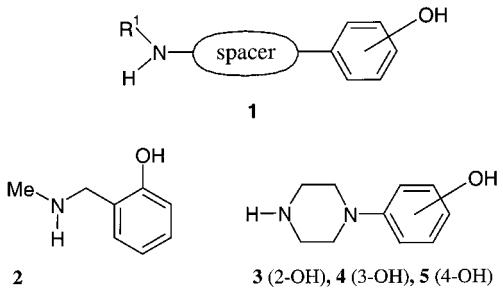

Figure 1.

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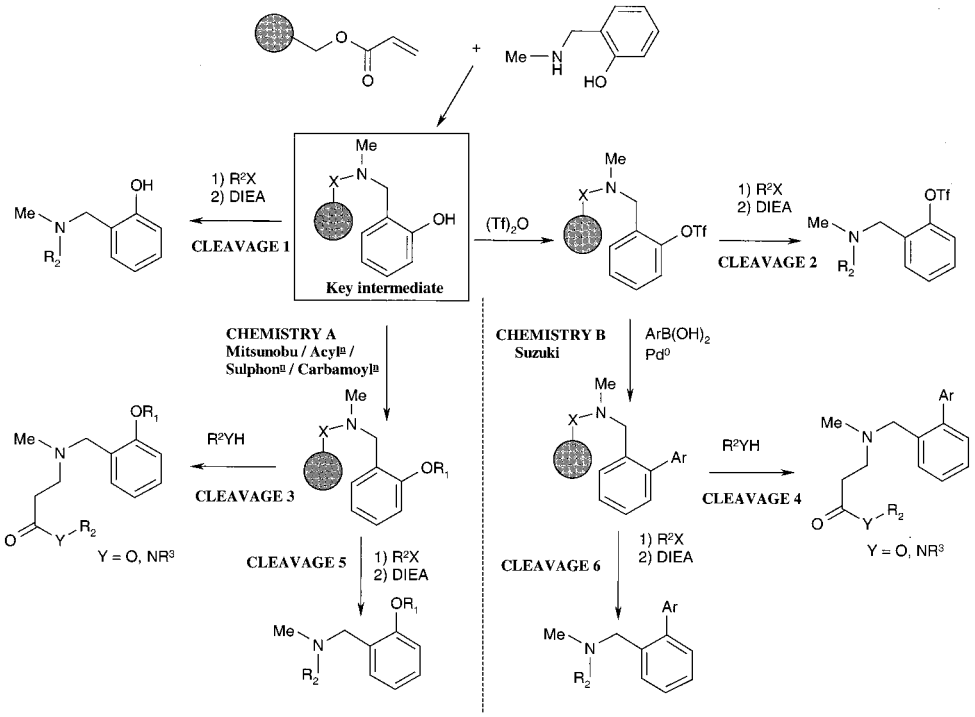
Scheme 1[a]



[a] X = $CH_2CH_2CO_2CH_2$.

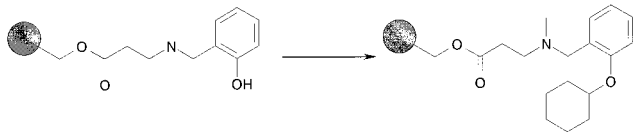
chemical strategy, each of the 13 scaffolds generated 234 compounds, so the total was therefore 3042 tertiary amines. The use of Irori MicroKans allowed us to minimize the number of individual reactions necessary for the synthesis of 3042 discrete compounds. We chose to use REM resin with a high loading (1.85 mmol/g) to maximize the mass of material that could be obtained from ~35 mg of resin in each MicroKan.

Method Development

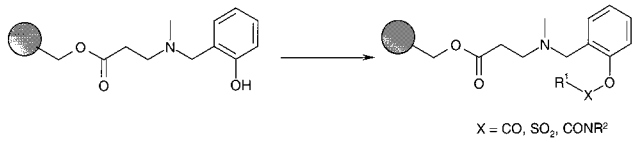
N-methyl-(2-hydroxy)benzylamine was the scaffold of choice for the majority of the method development work because it was commercially available and, because of the presence of the phenol in the sterically hindered ortho position, was likely to be one of the most synthetically challenging. The first step was a Michael addition in which the secondary amine nitrogen of the scaffold starting material was added to REM resin (1.8 mmol/g), using standard conditions (4 equiv of scaffold, *N*,*N*-dimethylformamide (DMF), 20 °C, 18 h). The resin-bound free phenol group was then functionalized using one of five different types of chemistry: (1) Mitsunobu reaction, (2) acylation, (3) sulfonation, (4) carbamoylation, or (5) triflation followed by Suzuki coupling.

The Mitsunobu reaction [chemistry A in Scheme 1] was optimized for the ortho-substituted benzylamine scaffold in combination with cyclohexanol as the alcohol, since this was considered to be a less favorable combination (ortho-substituted phenol plus secondary alcohol) (Scheme 2). Triphenylphosphine was preferred over tributylphosphine, and 1,1′-(azodicarbonyl)dipiperidine (ADDP) gave better results than diethyl azodicarboxylate (DEAD) or diispropyl azodicarboxylate (DIAD). As solvent, tetrahydrofuran (THF) gave better results than dichloromethane (DCM) or THF/DCM (1:1). The best conditions were found to be the alcohol (3 equiv), PPh_3 (3 equiv), and ADDP (3 equiv) in THF at 20 °C for 18 h. When these conditions were used, 16 alcohols were successfully coupled and were selected for the library.

Scheme 2



Scheme 3



Acylation, carbamoylation, or sulfonation gave esters (R^1CO_2Ar), carbamates ($R^1R^2NCO_2Ar$), or sulfonates (R^1SO_2Ar), respectively [chemistry A in Scheme 1]. Again, the acylation and sulfonation reactions were optimized for the ortho-substituted benzylamine scaffold using acetic anhydride and 4-benzenesulfonyl chloride, respectively (Scheme 3). The best conditions were found to be the acylating, sulfonating, or carbamoylating agent (10 equiv) and *N*,*N*-diisopropylethylamine (DIEA) (10 equiv) in DCM at 20 °C for 18 h.

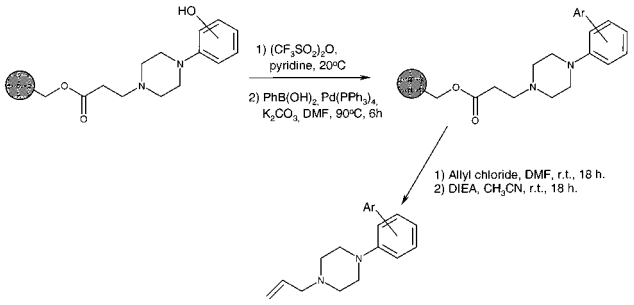
By conversion of the phenol to a triflate followed by a Suzuki coupling using an aryl boronic acid, a biaryl group (Ar−Ar) was formed [chemistry B in Scheme 1]. Aryl triflates are known to be less reactive substrates in palladium-catalyzed Suzuki couplings than aryl halides.[4] However, we were keen to use aryl triflates because of the fact that they are readily accessible from resin-bound phenols. The triflation and Suzuki reactions were optimized for scaffolds

Table 2. Analysis of Yields and Purities for Library Compounds

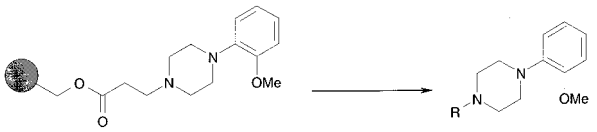
no.	catalyst[a]	base[b]	temp (°C)	solvent	time (h)	isomer	yield[c] (%)	purity[d] (%)
1	$Pd(PPh_3)_4$	3 M K_2CO_3	90	DMF	6	ortho	37	92
2	$Pd(PPh_3)_4$	3 M K_2CO_3	90	DMF	6	meta	25	76
3	$Pd(PPh_3)_4$	3 M K_2CO_3	90	DMF	6	para	31	84
4	$Pd(PPh_3)_4$	3 M K_2CO_3	90	DMF	15	ortho	20	87
5	$Pd(PPh_3)_4$	3 M K_2CO_3	70	DMF	15	ortho	33	63
6	$Pd(PPh_3)_4$	Et_3N (20 equiv)	90	DMF	24	ortho	*e*	46
7	$Pd(PPh_3)_4$	K_3PO_4	90	DMF	15	ortho	*e*	53
8	$Pd(PPh_3)_4$	$CsCO_3$	90	DMF	15	ortho	*e*	34
9	$Pd(OAc)_2$/*o*-Tol_3P	3 M K_2CO_3	90	DMF	15	ortho	*e*	36
10	$Pd_2(dba)_3$	3 M K_2CO_3	90	DMF	15	ortho	*e*	44
11	$Pd(PPh_3)_4$	Et_3N (20 eq)	90	toluene	15	ortho	*e*	47
12	$Pd(PPh_3)_4$	3 M K_2CO_3	90	TEGDE	6	ortho	38	78

[a] 10−20 mol %. [b] 2−4 equiv. [c] Estimated by ^1H NMR using a standard solution of maleic acid in DMSO-d_6. [d] HPLC (254 nm) purity of the biphenyl product. The remainder of the crude product is unreacted triflate. [e] Not determined because of low conversion (<60%).

Scheme 4



Scheme 5



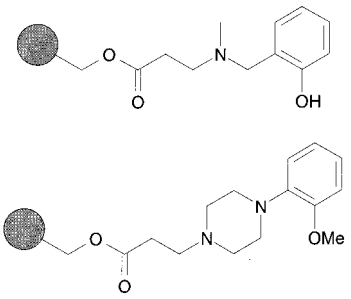


Figure 2.

3−**5** with phenylboronic acid (Scheme 4). Optimal conditions for the formation of the triflate were found to be 2-[*N*,*N*-bis(trifluoromethylsulfonyl)amino]pyridine and triethylamine in DCM at 20 °C for the meta- and para-substituted phenols and trifluoromethanesulfonic anhydride in pyridine at 20 °C for the more sterically hindered ortho-substituted phenols. The triflate was also quaternized and eliminated to increase library size and diversity with little extra effort [cleavage 2 in Scheme 1].

Over 30 different reaction conditions were investigated for the Suzuki reaction, varying catalyst, base, solvent, temperature, and reaction time. Unfortunately none of these conditions worked when the Irori MicroKans were used. Presumably the access of some of the reagents to the interior of the Kan is inhibited. The aqueous base is sufficiently viscous that it does not easily penetrate the mesh walls even with vigorous stirring. Nonaqueous homogeneous conditions using an organic base, Et_3N, were not successful either. Fortunately, when a MultiSyntech Syro II robot was used, the coupling was successful. It was found that $Pd(PPh_3)_4$ was better than $Pd(OAc)_2$ or $Pd(dba)_2$ as the catalyst (Table 2). Aqueous K_2CO_3 was preferred as the base over Et_3N, K_3PO_4, or Cs_2CO_3. The best conditions were found to be the boronic acid (3equiv), $Pd(PPh_3)_4$ (20 mol %), 3 M aqueous K_2CO_3 (2.8 equiv) in DMF at 90 °C for 6 h. This is the first example of a solid-phase Suzuki coupling involving a resin-bound triflates being used for library synthesis.[5] Four boronic acids were successfully coupled and were selected for the library. Besides modification of the phenol group, we considered it worthwhile to also cleave the underivatized phenol, since a number of known CNS drugs, such as several μ opioids, contain a free phenol group [cleavage 1 in Scheme 1].

Quaternization and Cleavage

Finally the second site of diversity was introduced by quaternization of the nitrogen using a reactive alkyl halide, and the compound was cleaved by Hofmann elimination (cleavages 5 and 6 in Scheme 1). Scaffold **3** was used as a model to test the feasibility of a number of potential quaternizing reagents (Scheme 5). This piperazine was chosen because it was thought to be the most problematic of the library scaffolds, since it is sterically hindered and has the possibility of nonregioselective quaternization at either of the two piperazine nitrogens. However, six quaternizing reagents, one of which was methyl iodide, successfully provided acceptably pure products in >40% overall yield and were selected for the library.

In addition to the quaternization and Hofmann elimination cleavages associated with REM resin methodology, the substrates gave amides and methyl esters by cleavage of the ester linkage to the resin [cleavages 3 and 4 in Scheme 1]. We found this divergent cleavage strategy to be an efficient way of expanding the diversity of the library while minimizing the number of individual reaction steps. The two resin-bound substrates shown in Figure 2 were used for the initial studies.

Several methods were assessed for the cleavage of the ester bond to give a methyl ester (Scheme 6). The favored method was heating the loaded MicroKan in methanol/triethylamine

Scheme 6

Scheme 7

(9:1) at 50 °C. This method gives very clean products in acceptable yields (~50%), with a simple workup consisting only of evaporation of the solvents.

Resin-bound esters were cleaved to give two amides using benzylamine and diethylamine as the amine components (Scheme 7). This was carried out by an aluminum chloride promoted aminolysis procedure,[3] which was shown to be compatible with Irori MicroKans [$AlCl_3$ (3 equiv), DCM, 20 °C, 20 min]. After cleavage, compounds were purified by silica gel solid-phase extraction (SPE).

Computational Analysis

Molecular properties for each compound in the library were calculated using the Chem-X ChemStat module: molecular weight (MW), number of H-bond acceptors (H-acc), number of H-bond donors (H-don), and number of rotatable bonds (RotB). The lipophilicity (clogP) was calculated using CLOGP3, PCModels version 4.34 (Daylight Chemical Information Systems, Inc., Irvine, CA). A QSAR-derived estimate of the logarithm of the brain/blood distribution (cLogBB) was also calculated for each compound.[6] Regression analyses between simple molecular descriptors and published brain/blood distribution ratios for a variety of drug and nondrug molecules[1] showed that a reasonable correlation could be derived:

$$\log BB = (0.28)(\text{clogP}) - (0.167)(\text{H-don}) - (0.032)(\text{MW}) + 0.16$$

$$n = 60; \quad r^2 = 0.80; \quad F = 75.8$$

The log BB is the logarithm of the brain/blood distribution ratio, clogP is the calculated logarithm of the octanol/water partition coefficient, H-don is the number of hydrogen bond donors, MW is the molecular weight, n is the number of compounds studied, r^2 is the correlation coefficient, and F is the F-test for statistical significance.

The histograms shown in Figures 3−8 graphically represent the molecular property distribution of the library. The property ranges exhibited by a majority of the CNS drugs (i.e., 66% of CNS drugs) are within the stated property limits.

Overall, the property distributions for the molecules in the library agree very favorably with the property distributions observed for known CNS drugs. The predicted brain/blood distribution ratios are particularly encouraging and indicate a high probability that the compounds in this library will penetrate into the CNS.

Library Production

A large batch of high-loading REM resin (150 g) was made from Merrifield resin and cesium acrylate. Most of the scaffolds were initially prepared as hydrochloride or hydrobromide salts, so they were converted to the free amine before loading to REM resin. A batch of ~12 g of each resin-bound scaffold was prepared. Of this, ~7 g was loaded by slurrying in DCM/DMF into Irori MicroKans (35 mg per kan; preformatted kans in 96-well plates). All Mitsunobu, acylation, and quaternization reactions were conducted in

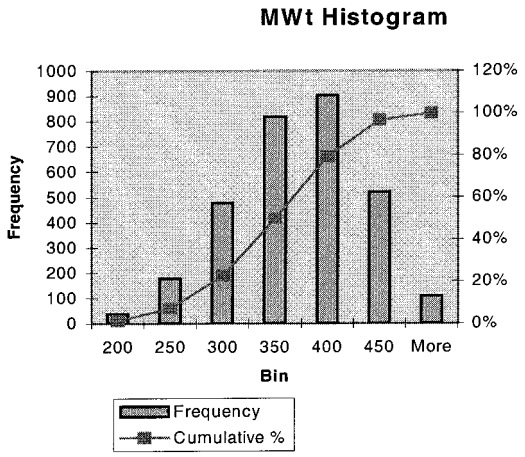


Figure 3. 66% of CNS active drugs have MW between 125 and 425.

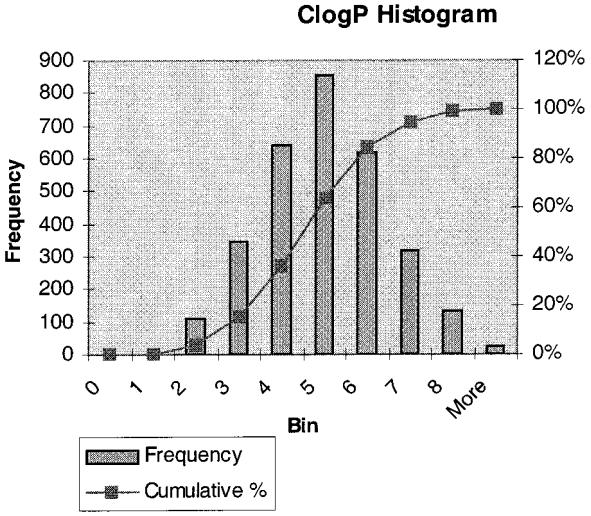


Figure 4. 66% of CNS active drugs have clogP between 1.5 and 6.5.

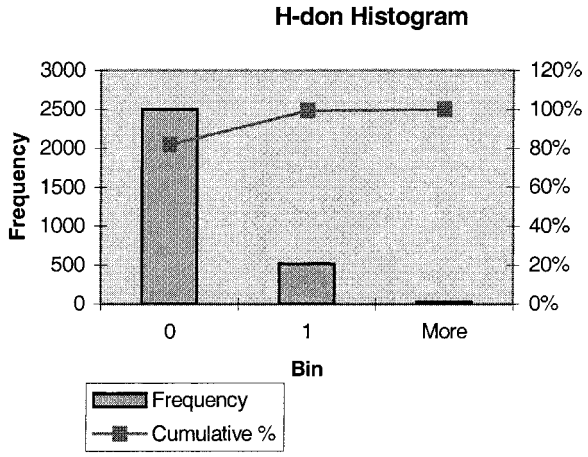


Figure 5. 66% of CNS active drugs have zero to two H bond donors.

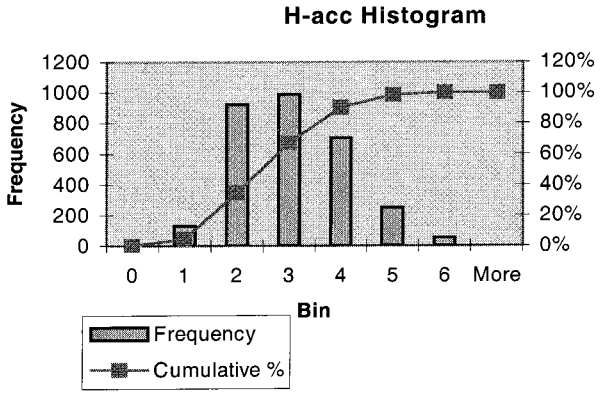


Figure 6. 66% of CNS active drugs have one to two H bond acceptors.

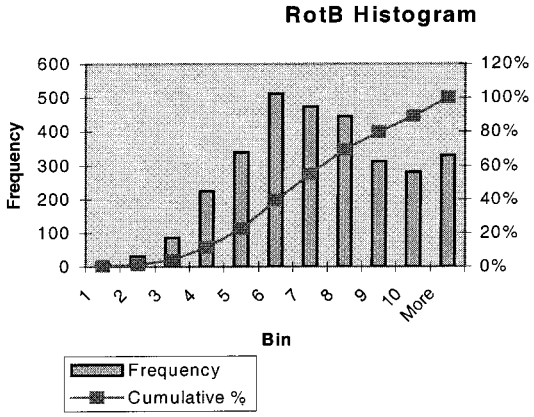


Figure 7. 66% of CNS-active drugs have zero to seven rotatable bonds.

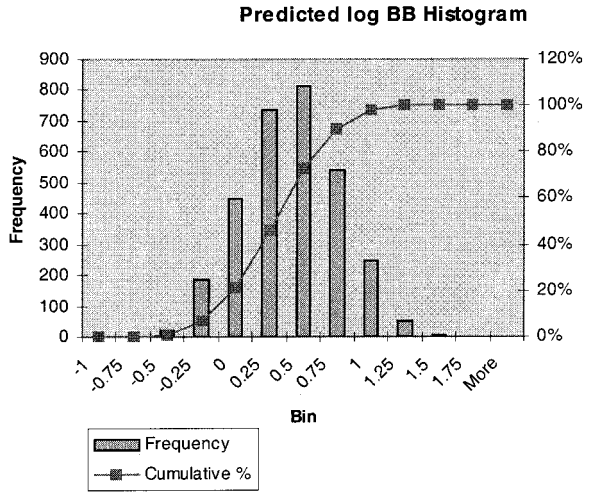


Figure 8. 66% of CNS-active drugs have cLogBB between −0.74 and 0.77.

MicroKans within quickfit 250 mL conical flasks (magnetic stirring or orbital shaking). Approximately 3 g of each of the resin-bound scaffolds was converted into the aryl triflate and then was loaded into Syro II reactors for the Suzuki reaction. Heating and intermittent stirring were then provided using the Syro II robot reaction block.

The cleavage was done using two Irori 96-vessel cleavage stations. Because heating to 50 °C was required, the methanolysis cleavages were done using a STEM block rather than the Irori cleavage station. The aminolysis cleavages were done in 4 mL glass vials, and the crude products were purified by SPE.

Library Analysis

The library was analyzed using a combination of flow injection electrospray MS, reverse-phase HPLC, and ^{1}H NMR. All 3042 samples were analyzed by MS, 608 samples (20%) by HPLC, and 74 samples (2.4%) by NMR. The yield for the 74 NMR samples was determined gravimetrically (see Supporting Information). Out of a total of 3042 compounds, 2725 were found to be present by MS (90% of the library) (Table 3). A confirmatory mass spectrum was regarded as one in which the expected molecular ion is at least 5% of the base peak intensity.

The average purity of the samples was found to be 69%, based on integration of the largest peak in the HPLC profile. The yield of product was an acceptable 11.2 mg (approximately 0.033 mmol), which should provide enough material for both the screening and the hit followup studies. The data suggest that the Mitsunobu and acylation chemistries gave slightly higher yields and purities of product than the Suzuki chemistry.

HTS Screening

One of the targets at which the library was screened was the $GlyT_2$ transporter. A number of compounds were found to be active at 10 μM and were resynthesized for IC_{50} determination. The most potent of these was compound **6** (Figure 9). The crude sample of **6** from the HTS well gave an IC_{50} of 1.3 μm and was determined to have a purity of 77% by LC−MS. The compound was purified by reverse-phase HPLC to give a sample that was >95% pure by ^{1}H NMR and LC−MS analysis. The IC_{50} of the purified material was essentially identical to that of the crude library product (1.0 μM), indicating that the purity of the library was adequate for finding useful hits from HTS. Compound **6** possessed good selectivity over the $GlyT_1$ transporter, being inactive at 100 μM. As well as possessing interesting biological activity, the physicochemical properties of **6** are consistent with a CNS-drug-like profile. Being a hit from a combinatorial library, the synthetic methodology for rapid SAR exploration and analogue synthesis already exists, so **6** was selected for an optimization program.

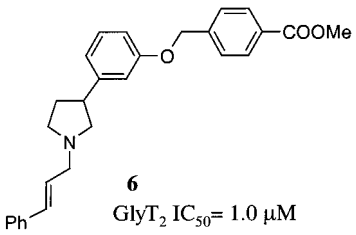
Summary

A 3042-compound screening library was synthesized using REM resin methodology and $AlCl_3$-assisted aminolysis, in combination with IRORI MicroKans. The novelty and diversity of the library were enhanced by using multiple scaffolds (many proprietary) and a divergent strategy involving five different types of chemistry (Mitsunobu, acylation, sulfonation, carbamoylation, Suzuki). Computational analysis suggests that the compounds are likely to have favorable properties for CNS penetration. This is an "information-rich" library in the sense that it should be possible to gain much SAR information around any interesting hits, from other compounds in the library. The discovery of a novel structure

Table 3. Analysis of Yields and Purities for Library Compounds

	chemistry A[a]	chemistry B[b]	overall
total no. of compounds with MS intens ≥ 5%[c]	2228	497	2725
total no. of compounds with MS intens < 5%	229	88	317
overall % of compounds with MS intens ≥ 5%	91	86	90
average HPLC % purity[d]	70	66	69
average yield (mg)[e]	11.5	10.4	11.2
average yield (mmol)	0.035	0.029	0.033

[a] Chemistry A = Mitsunobu, acylation, sulfonation, carbamoylation. [b] Chemistry B = Suzuki coupling. [c] The expected molecular ion is at least 5% of the base peak intensity. [d] Average purity of 20% of the samples (every fifth sample) with UV detection at 214 nm. [e] Products contained small residual amounts of DIEA salts.



6

$GlyT_2$ IC_{50}= 1.0 μM

MWt.:	427
cLogP:	6.24
H-don	0
H-acc	4
RotB:	9
cLogBB:	0.5

Figure 9.

with interesting biological activity against the GlyT2 transporter demonstrates that this library is of practical value for high-throughput screening.

Experimental Section

Synthesis of REM Resin. Six individual batches were prepared using the following procedure and then mixed thoroughly to ensure homogeneity. To a mixture of Cs_2CO_3 (81.6 g, 250 mmol) and KI (4.17 g, 25 mmol) in DMF (250 mL) was added acrylic acid (18 mL, 260 mmol) at 20 °C. The mixture was stirred for 15 min, then Merrifield resin (25 g, 50 mmol, Polymer Laboratories, loading 2 mmol/g) was added and the mixture was heated at 80 °C for 18 h. After the mixture was cooled, the resin was collected by filtration and was washed with 250 mL portions of water, DMF, water/DMF (1:1), DMF, water/DMF (1:1), DMF, CH_3OH, DCM, CH_3OH, and DCM. The six resin batches were combined in DCM, stirred, filtered, and then dried under vacuum at 40 °C.

General Procedure for the Michael Addition. To 10 g of REM resin (loading 1.67 mmol/g, 16.7 mmol) was added a solution of the relevant amine scaffold (67.7 mmol) in DMF (100 mL). The flask was then sealed and placed on an orbital shaker for 48 h at 20 °C. The resin was then removed by filtration and washed with DMF (20−30 mL). The resin was then washed with more DMF, (3 × 150 mL), then with DCM (3 × 200 mL), CH_3OH (100 mL), then with more DCM (2 × 100 mL), and finally with CH_3OH (100 mL). It was then dried in vacuo at 40 °C for 5 h. IR, cm^{-1}: 3393 (OH), ∼1730 (C═O sat.), ∼1170 (C−O).

General Procedure for the Mitsunobu Coupling. A set of 54 MicroKans was placed in a 250 mL conical flask. Each MicroKan contained approximately 40 mg (0.052 mmol) of resin so that the total amount of phenol substrate in the reaction was approximately 2.8 mmol. Anhydrous THF (80 mL) was added to the flask, which was then vacuum-degassed (2×) and flushed with nitrogen. The relevant alcohol (8.4 mmol), triphenylphosphine (2.1 g, 8.4 mmol), and ADDP (2.02 g, 8.4 mmol) were added, and the reaction was stirred for 18 h at 20 °C. The MicroKans were then collected by filtration, washed alternately with CH_3OH and DCM (3 × 100 mL), and then dried in vacuo. A small sample of resin was removed and tested with $FeCl_3$ solution. A slight blue coloration indicated residual phenol, so the above sequence was then repeated a second time to drive the Mitsunobu reaction to completion. The $FeCl_3$ test was now negative.

General Procedure for the Acylation, Sulfonation, and Carbamoylation. A set of 54 MicroKans was placed in a 250 mL conical flask. Each MicroKan contained approximately 40 mg (0.052 mmol) of resin so that the total amount of phenol substrate in the reaction was approximately 2.8 mmol. Anhydrous DCM (100 mL) was added to the flask, which was then vacuum-degassed (2×) and flushed with nitrogen. DIEA (3.7 g, 28 mmol) and the acylating or sulfonating agent (28 mmol) were added, and the reaction was agitated for 18 h at 20 °C using an orbital shaker. The MicroKans were then collected by filtration, washed alternately with CH_3OH and DCM (3 × 100 mL), and then dried in vacuo.

General Procedure for Triflation of Ortho-Substituted Phenols. Triflic anhydride (0.12 mL, 0.7 mmol) was added dropwise at 0 °C into a suspension of resin-bound phenol (100 mg, 0.14 mmol) in anhydrous pyridine (2 mL). The reaction suspension was allowed to warm to 20 °C over 1 h and then continued with agitation overnight using an orbital shaker. The resin was drained, washed with DCM (3 × 10 mL), 5% aqueous sodium carbonate (2 × 10 mL), 5% triethylamine in DCM (2 × 10 mL), DCM (3 × 10 mL), and CH_3OH (3 × 10 mL), and dried in vacuo at 40 °C.

General Procedure for Triflation of Para- and Meta-Substituted Phenols. To a suspension of resin-bound phenol (1 g, 1.39 mmol) in anhydrous DCM (10 mL) was added 2-[*N*,*N*-bis(trifluoromethylsulfonyl)amino]pyridine (1.5 g, 4.17 mmol) followed by a dropwise addition of triethylamine (0.6 mL, 4.17 mmol). The reaction suspension was agitated for 3 h at 20 °C, washed with DCM (3 × 30 mL), water (2 × 30 mL), 5% aqueous sodium carbonate (2 × 30 mL), and CH_3OH (3 × 30 mL), and dried in vacuo at 40 °C.

General Procedure for the Suzuki Reaction. Resin-bound aryltriflates (120 mg, 0.138 mmol) were added to glass

reactors (5 mL) of a MultiSynTech SyRo I synthesis robot. The resin within each of the reactors was suspended with degassed DMF (3 mL) followed by addition of the boronic acid (0.414 mmol), $Pd(PPh_3)_4$ (36 mg, 0.028 mmol), and 3 M K_2CO_3 (0.12 mL). The reaction was carried out under an argon atmosphere at 90 °C over 6 h with intermittent stirring (10 s every 30 min). The reactor block was allowed to cool to 20 °C, and the resin was drained, washed with DMF (3 × 2 mL), DCM (3 × 2 mL), and CH_3OH (3 × 2 mL), and dried in vacuo at 40 °C.

General Procedure for the Quaternization. A set of 126 MicroKans was placed in a stoppered conical flask. Each MicroKan contained approximately 40 mg (0.052 mmol) of resin so that the total amount of quaternization substrate in the reaction was approximately 6 mmol. DMF (200 mL) was added to the flask, which was then vacuum-degassed (2×). Methyl iodide (3.8 mL, 61 mmol) was added, and the reaction mixture was agitated for 18 h at 20 °C on an orbital shaker. The MicroKans were then collected by filtration, washed alternately with CH_3OH and DMF (3× each), then washed alternately with CH_3OH and DCM (3× each), and finally washed with DCM (2×) and then dried in vacuo. Some of the quaternization reagents employed in the library synthesis required higher temperature (50 °C) for successful reaction. All other conditions remained identical.

General Procedure for the Hofmann Cleavage. The Irori MicroKans were each immersed in a 0.3 M solution of diisopropylethylamine in acetonitrile (1 mL) within an Irori cleavage block. The MicroKans were agitated for 5 min and then left standing overnight at 20 °C. The cleavage solution was filtered under vacuum into a 96-well plate, and the resin was washed with DCM (1 mL per can). The combined filtrates were concentrated to dryness using a vacuum centrifuge (Savant SpeedVac).

General Procedure for the Esterolysis Cleavage. Each MicroKan containing approximately 40 mg (0.052 mmol) of resin was separately placed in a STEM block reaction vessel with a magnetic follower. Methanol (3 mL) and triethylamine (0.3 mL) were added, and the vessel was vacuum-degassed (3×). The reaction mixture was then heated at reflux with magnetic stirring for 18 h. The reaction mixture was cooled to 20 °C, and the solution was collected by pipet, rinsing the MicroKan with methanol. The resulting solution was concentrated under reduced pressure to yield the methyl ester.

General Procedure for the Aminolysis Cleavage. The relevant amine (32 mmol) was added to a stirred suspension of aluminum chloride (8 mmol) in 240 mL of DCM, and the mixture was stirred at 20 °C for 20 min. Each MicroKan containing approximately 40 mg (0.052 mmol) of resin was placed in a 7 mL screw-capped vial, and to each vial was added a total of 3 mL of the reaction mixture. The vials were then agitated for 24 h at 20 °C on an orbital shaker. A total of 0.2 mL of 2 M K_2CO_3 solution was added to each reaction, and then each vial was agitated rapidly for 30 s on a Vibromix shaker. The DCM solutions in each vial were transferred to 100 mg silica gel SPE columns (pretreated with 2% CH_3OH in DCM) and the columns eluted with 3 mL of 5% CH_3OH in DCM. Eluents were evaporated under reduced pressure to afford products. A total of 78 IRORI MicroKans were cleaved using this procedure.

Sample Handling. The crude cleavage products were collected and evaporated to dryness in 2 mL Beckmann microtiter plates. DMSO (400 μL) was added to each product using a Hewlett-Packard Multiprobe. After standing at ambient temperature for 24 h, each product/DMSO mixture was aspirated and redispensed into the same well to ensure a homogeneous solution. The contents of each well were transferred to a corresponding well in a 1 mL microtiter plate. Each original well was rinsed with 400 μL of DMSO, and these solutions were added to the main solutions in the storage plates. The residue in each well of the 2 mL plate was dissolved in 500 μL of CH_3CN, and these solutions were transferred to 1 mL microtiter plates and submitted for electrospray MS. Every fifth sample (70 μL) was transferred manually to 1 mL microtiter plates, which were submitted for reverse-phase HPLC after evaporation of the DMSO. Finally, 50 μL of each solution was transferred to each of two 1 mL microtiter plates and the storage plates were evaporated to dryness. In total, 200 microtiter plates were manipulated during the preparation of screening and analytical samples.

Compound 6. 6 was obtained following generic procedures: (a) Michael addition of 3-(4-hydroxyphenyl)pyrrolidine to REM resin (40 mg, 0.052 mmol); (b) Mitsunobu reaction with methyl 4-(hydroxymethyl)benzoate; (c) quaternization with cinnamyl bromide; (d) Hofmann elimination using diisopropylethylamine. The crude product (23.1 mg, 81%) was purified by reverse-phase HPLC carried out on a Gilson system using LUNA C18 column (5 μm, 60 mm × 21.2 mm) under gradient conditions (10 min, gradient of 10% acetonitrile/90% water/0.1% TFA to 90% acetonitrile/10% water/0.1% TFA) at a flow rate of 5 mL/min to obtain 15.4 mg (54%) of compound **6**. ^{1}H NMR ($CDCl_3$): δ 2.14 (m, 0.5H), 2.38 (m, 0.5H), 2.57 (m, 0.5H), 2.86 (m, 0.5H), 2.98 (m, 0.5H), 3.14 (m, 0.5H), 3.42 (m, 0.5H), 3.51 (m, 0.5H), 3.72 (m, 1H), 3.92 (s+m, 6H), 4.03 (m, 1H), 5.11 and 5.15 (s, 2H), 6.31 (m, 1H), 6.75 (m, 1H), 6.83 (m, 3H), 7.26 (t + t, 1H), 7.38 (m, 3H), 7.40 (d, 2H), 7.49 (d + d, 2H), and 8.05 (d + d, 2H). MS (ES): *m*/*z* 428.2 (MH^+). LC−MS *m*/*z*: 428.2 (MH^+, >99%).

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Supporting Information Available. A table listing the weights of 74 library members. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Data were obtained by searching the World Drugs Index (Derwent Information, Ltd.) for currently marketed compounds with a tradename, which are coded for CNS activity.

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